REMARKS

Status of the Claims

Claims 1-18 and 29-30 are currently under consideration.

The withdrawal of various previous rejections and objections as listed at pages 2-3 of the Office Action is noted with appreciation.

Sequence Listing Requirements

In accordance with the Notice to Comply, applicant submits herewith a paper copy of the sequence listing submitted as a .txt file on the EFS system on July 7, 2008. The paper copy submitted herewith and the .txt file previously submitted are the same. This paper copy of the sequence listing adds no new matter to this application.

Claim rejections – 35 U.S.C. § 103

The present claims are directed toward modified Fab' fragments to which at least one effector molecule is attached, characterized in that the heavy chain in the fragment is not covalently bonded to the light chain, and further in that *both* the interchain cysteine of C_L *and* the interchain cysteine of C_H1 have been replaced with another amino acid, and the hinge region contains one or two cysteines. Claim 1 previously was amended to recite that at least one effector molecule is a 5,000 to 40,000 KDa PEG or a PEG derivative. It has been found that despite the absence of any covalent linkage between the heavy and light chain and the attachment of one or more effector molecules, the fragments of the invention as recited in the present claims perform comparably with wild type fragments in a number of in vitro and in vivo tests. Surprisingly, these novel fragments have the same affinity for antigen and similar in vivo and in vitro stability as wild type fragments (specification, at paragraph spanning pages 2-3).

The claims stand rejected as obvious under two sets of references; these two grounds of rejection will be addressed in turn.

Chapman et al. in view of Humphreys et al.

The rejection of the claims as obvious over Chapman et al. (Nature Biotechnology, 17:780-783, 1999) in view of Humphreys et al. (J. Immun. Methods, 209:193-202, 1997) is respectfully traversed. Chapman et al. discloses that Fab' fragments with a PEG attached at the hinge region have improved half-lives without loss of antigen-binding affinity. Chapman does not teach or suggest that any modification should be made to either the light chain or the heavy chain of the Fab' fragment. Fig. 2 of the Chapman et al. disclosure specifically shows an intact covalent bond between the light and heavy chains, thus teaching away from the presently claimed invention.

Humphreys et al. is directed to the formation of dimeric Fab's in E. Coli., because in clinical applications it is often desirable to have the increased effective binding affinity afforded by a dimeric Fab' (Humphreys et al. at p. 193, second column, lines 5-7) The disclosure examines the effects of various parameters, including hinge size and isotype, presence of interchain disulphide bond, Fab' expression levels, tail piece sequences, and growth conditions. Noticeably absent from the parameters investigated by Humphreys et al. is the presence or absence of effector molecules of any kind. Further, Humphreys et al. found that the unmodified γ1 hinge gave the greatest $F(ab')^2$ yield in vivo (p. 198, second column, lines 41-43). Thus, to the extent Humphreys et al. teaches anything about Fab' modifications, Humphreys et al. teaches *away* from modification of the hinge region.

Applicants respectfully submit that the rejection is based on impermissible hindsight, using the applicants' invention as a template to piece together unrelated elements from other disclosures, in a manner not taught or suggested by the prior art. Chapman et al. teaches PEGylated Fab' fragments with intact heavy chain – light chain bonds. Humphreys et al. teaches modification of Fab' fragments having no effector molecules in order to prepare F(ab')2 fragments. These two endeavors have different goals. There is no reason why one interested in PEGylated antibody fragments would have turned to the Humphreys et al disclosure. Nor would Humphreys et al.'s disclosure of modified hinge regions and the lack of a light-chain-heavy chain bond have suggested anything to the skilled artisan about the effects thereof on PEGylation. Nor would one skilled in the art have preferentially selected these aspects of the Humphreys et al.

disclosure rather than any of the other parameters studied in the disclosure, i.e., effect of hinge size, Fab' expression levels, tailpiece sequences, and growth conditions. There is simply no reason why one studying the Chapman et al. disclosure relating to PEGylated fragments, would have turned to the Humphreys et al. article, and would have randomly focused on two out of six parameters reviewed therein, to arrive at the presently claimed invention. Nor would there have been any expectation that Fab' fragments having both of the heavy and light chain cysteines replaced with another amino acid would have had the same antigen affinity as wild type fragments. It is therefore requested that this ground of rejection be withdrawn.

Singh et al. in view of Hsei et al. and Humphreys et al.

The rejection of the claims as obvious over the combination of Singh et al. (Analytical Biochemistry, 304(2): 147-156, May 15, 2002, in view of Hsei et al. (WO 98/37200, 8/27/98) and Humphreys et al., cited above, is respectfully traversed.

The combination of the Singh, Hsei, and Humphreys references does not teach or suggest the present invention. The disclosures of each of these references will be discussed first singly, then in combination.

(a) The Singh reference

Singh et al. teaches a method of labeling whole antibodies, with non-selective selenol-catalyzed reduction employing labels such as biotin-PEO-maleimide complex having a formula weight of 525.6. The catalyzed reduction resulted in the addition of seven labels in less than 5 minutes. The labeled antibodies of the Singh disclosure are different from the conjugated antibody fragments of the present invention in two important respects.

First, conjugating effector molecules to a Fab' fragment presents different challenges than labeling a whole antibody. Because the whole antibody has a greater area, particularly in the constant region, over which the labels may be distributed, the properties of the whole antibody may be less affected by the process than a Fab' fragment would be. An Fab' fragment, having no constant region extending beyond the hinge region, would be expected to be more subject to destabilizing effects of large effector

molecules; one skilled in the art would have expected the heavy and light chains to be pulled apart if there were one or more effector molecules attached to them and there was no covalent bond between them. Thus, results obtained with placement of effector molecules on whole antibodies can not necessarily be used to predict the effect of conjugation of effector molecules to antibody fragments. The teachings of Singh relating to conjugated whole antibodies would not teach or suggest to one of ordinary skill in the art of conjugated antibodies and antibody fragments that similar results could be achieved with conjugated fragments having no covalent bond between the heavy and light chains and no constant region beyond the hinge.

Second, the labels used in Singh were very different from the effector molecules recited in the present claims. The label molecules of Singh were biotin-PEO-maleimide having a formula weight of 525.6 (Singh, page 149, left column, lines 5-9). By contrast the effector molecules described in the present application are PEG or PEG derivatives having a molecular weight of at least about 5,000, effectively a ten-fold increase, and the effector molecules used in the examples as described in the present specification were much larger. One of ordinary skill in the art would appreciate that the results achieved by Singh using *small* molecules with *whole* antibodies could not be used to predict the results that would be achieved using *large* effector molecules with Fab' antibody *fragments*. The recitation of large effector molecule size and Fab' antibody fragments in the present claims distinguishes these claims over Singh.

Contrary to the Examiner, the teachings of the secondary references Hsei and Humphreys do not make up for the deficiencies of Singh, as explained below.

(b) <u>The Hsei reference</u>

Hsei teaches the following (emphasis added):

- *F(ab')*^{2} fragments having no more than about *two* polymer molecules, wherein every polymer molecule is attached to a cysteine residue in the light or heavy chain that would ordinarily form a disulfide bridge linking the light and heavy chains (p. 23, lines 4-8; p. 24, line 24 – p. 25, line 32; p. 33, line 33 – p. 35, line 6).

- Fab, Fab', and Fab'-SH fragments having *no more than one* polymer molecule, wherein the polymer molecule is coupled to a cysteine residue in the light or heavy chain that would ordinarily form a disulfide bridge linking the light and heavy chains (p. 23, lines 9-14; p. 25, line 33 – p. 27, line 7; p. 35, line 7 – p. 36, line 21).
- Fab, Fab', and Fab'-SH fragment conjugates containing *more than one polymer* molecule, wherein *every* polymer molecule in the conjugate is *attached to the hinge region* (p. 23, line 15 – p. 24, line 23; page 31, line 25 – p, 33, line 32);
- Fab, Fab', and Fab'-SH fragment conjugates containing *no more than one* polymer molecule, wherein the polymer molecule in the conjugate is *attached to the hinge region* (p. 27, line 8 – p. 28, line 4);
- Fab, Fab', and Fab'-SH fragment conjugates containing *no more than one* polymer molecule (p. 36, line 22 – p. 37, line 31).

At page 28, line 32 – page 30, line 5, Hsei generally discusses conjugates of antibody fragments with more than one PEG molecule, but does not discuss the type of fragments or where on the fragments the PEG molecules are attached. At page 30, line 6 – page 31, line 24, Hsei discusses Fab, Fab', Fab'-SH and $F(ab')^2$ fragments with more than one PEG molecule, but does not discuss where the PEG molecules are attached.

The present claims recite, inter alia, that both the interchain cysteines are replaced by another amino acid. By comparison, Hsei et al. teaches that the disulfide bridge is to be avoided by substituting another amino acid, such as serine, for the corresponding cysteine residue in the opposing chain, *only* for those embodiments where the polymer molecule is bonded to the corresponding cysteine residue *in the heavy or light chain* (page 23, line 9-14; page 24, line 24 – page 27, line 7)). Thus, even when one of the cysteines in the heavy or light chain is replaced, the other must be retained, because it forms the site of attachment of the polymer molecule. In all the other embodiments in which the polymer molecule is bonded to the *hinge* region*, Hsei says nothing about avoiding the disulfide bridge* between the heavy and light chains (page 23, line 15 - page 24, line 23; page 27, line 8 – page 28, line 4). Pages 37-38 are silent about the site of

attachment of the polymer molecule or the presence or absence of the disulphide bond. Page 42 discusses avoidance of the disulphide bond so that a polymer molecule can be attached to the corresponding cysteine in the opposite heavy or light chain, indicating that that cysteine was not replaced. Pages 98-102 discuss various steps in the preparation of the antibody. Pages 104-105 discuss compositions and administration of the antibody fragments.

Thus, Hsei makes the statement about the disulphide bridge being avoided only in the context of describing those embodiments wherein a polymer molecule is attached to a cysteine that otherwise would have been part of the interchain disulphide bond; and Hsei never suggests that both the heavy and light interchain cysteines are to be replaced with another amino acid, nor does Hsei suggest any reason for doing so.

(c) <u>The Humphreys reference</u>

The Humphreys et al. reference is discussed above. As noted, the Humphreys et al reference teaches nothing about effector molecules in general or PEGylation in particular. Moreover, Humphreys et al. is specifically directed to using Fab' fragments to make $F(ab')^2$ fragments, which are excluded from the scope of the present claims.

(d) <u>The combination of references does not render the presently claimed invention obvious</u>

The Examiner states that "It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have produced anti-IL-8 Fab, Fab', Fab-SH and $F(ab')^2$ fragments in which both the interchain cysteines of the CL and CH1 have been mutated to serines and the produced anti-IL-8 Fab, Fab', Fab-SH and $F(ab')^2$ fragments comprise a modified hinge region containing one or two cysteines (e.g., SEQ ID No.: 1, 2 or 3) as well as a cysteine residue or residues engineered into a selected site or sites in the antibody fragment (i.e., in both the heavy and light chain constant regions) for PEGylation according to the selenol catalyzed reduction of disulfides as taught by Singh et al. for therapeutic benefit of inflammatory disorders.

This statement is respectfully traversed. First, the Singh reference teaches a process that is not site-selective for placement of one or more effector molecules on an antibody. Instead, Singh teaches that each and every cysteine residue on the antibody molecule is to be reduced so that an effector molecule can be attached. Yet the Chapman reference, discussed above, teaches away from the use of a process such as disclosed in Singh. Chapman et al. teaches that "Unfortunately, PEG-conjugated proteins, including antibodies, tend to lose biological activity when conjugated to multiple PEG molecules." (col. 1, third paragraph) Particularly in regard to Fab' fragments, Chapman et al. teaches that "However, their ability to bind antigen was reduced as more PEG was attached." (page 1, column 2. lines 16-17). Thus one skilled in the art would not apply the method of Singh to Fab' fragments as suggested by the Examiner, because it would be expected that the multiple PEGylation achieved with Singh would lead to a loss of biological activity, and particularly for Fab' fragments a reduction in the ability to bind antigen.

Nor would one combine Singh et al. with Hsei. Hsei teaches, inter alia, that *either* polymer molecules can be attached to a fragment hinge region, *or* the interchain disulfide bridge can be broken by substitution of another amino acid for one cysteine in the bridge and a polymer molecule then attached to the heavy or light chain of the other cysteine in the bridge. There would be no reason to combine Hsei, which teaches selective PEGylation, with Signh, which teaches total PEGylation.

As noted above, the Humphreys et al. reference teaches nothing about effector molecules of any type, and is directed to ways of using Fab' fragments to make $F(ab')^2$. Thus one seeking to provide PEGylated Fab' fragments would have no reason to look to the teachings of Humphreys et al.

One of ordinary skill in the art would not be motivated to combine Singh with either Hsei or Humphreys, because one of ordinary skill in the art would not think that the teachings of Singh relating to total PEGylation of whole antibodies with small labels would be applicable to the teachings of Hsei relating to selective PEGylation of antibody fragments with large effector molecules, or to the teachings of Humphreys et al. relating to methods of making $F(ab')^2$ fragments.

The Examiner concludes at page 7, lines 19-28, "Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to produce anti-

IL-8 Fab, Fab', Fab-SH and $F(ab')^2$ fragments comprising the cysteine containing hinge peptides of SEQ ID Nos:1-3 as taught by Humphreys and reduced using the selenol-catalyzed reduction of interchain disulfides to expose reactive thiols to which PEG molecules are attached…." To the contrary, there is no reason why one of ordinary skill in the art would have been motivated to combine these references, especially when one considers that whole antibodies of Singh and $F(ab')^2$ of Humphreys fragments are *not part of the present invention*. Singh teaches effector molecules on whole antibodies, not fragments, and complete PEGylation, not selective; Hsei teaches that only one interchain cysteine is replaced, not both, and only then when the effector molecules are to be attached at the opposite chain, not at the hinge; and Humphreys teaches non-PEGylated $F(ab')^2$ fragments.

In view of the foregoing, where there is no reason to combine the cited refrences, and where such a combination would not yield the claimed invention, it is respectfully requested that the rejection under 35 U.S.C. 103 be withdrawn.

Double patenting

The double patenting rejection based on claims 7 and 10 of U.S 6,642,356 is respectfully traversed. As stated in the '356 Abstract, the reference discloses a peptide sequence that can be used as hinge regions in proteins, where they can be covalently coupled to achieve dimeric structures, for example, as found in antibodies. Independent claim 5 of the '356 patent recites an antibody fragment comprising one polypeptide chain having the recited amino acid sequence; claim 7 dependent on claim 5 recites that the fragment is a Fab or Fab' fragment, and claim 10 dependent on claim 7 recites the fragment with one or more effector or reporter molecules attached to it.

First, it is respectfully pointed out that the amino acid sequence recited in the claims of the '356 patent is not the same as the amino acid sequences of the present application. Moreover, SEQ ID NO:1 of the '356 patent contains *four* cysteines, yet claim 1 recites that the hinge region contains *one or two* cysteines. Thus the claims of the '356 patent actually teach away from the presently claimed fragments.

Whether or not it would have been prima facie obvious for one to make a Fab' fragment with SEQ 1 of the '356 patent is irrelevant to the question of whether the presently claimed invention is obvious. Nor is it relevant whether the free cysteine thiols of '356 patent SEQ ID NO:1 are attached to PEG molecules, because the present claims encompass embodiments wherein there are free cysteine thiols that are not attached to PEG molecules. The '356 patent is solely directed to the use of particular peptides in the hinge region to create dimers. Thus one skilled in the art seeking to create Fab' fragments would not have looked to Humphreys '356. The present claims are not obvious variants of claims 7 and 10 of '356.

The present invention is based on applicants' surprising discovery that an Fab' fragment could be produced having affinity for antigen comparable to wild type antibody, yet have no disulfide bridge between the heavy and light chains, and have the cysteines of both the heavy and the light chains replaced with another amino acid, and have one or two cysteines n the hinge region. The '356 patent is silent as to the presence or absence of cysteine residues in the heavy and light chains. There would have been no motivation to one of skill in the art to combine the '356 patent with Chapman et al. or Humphreys et al., nor would such a combination have resulted in the presently claimed fragments, because nothing in any of these references suggests that it would be desirable to have an Fab' fragment with no interchain bonds between the heavy and light chains, and with both cysteines in the heavy and light chains replaced with another amino acid, and with the hinge region containing one or two cysteines.

CONCLUSION

As all points of rejection have been overcome, a Notice of Allowance is respectfully requested. The Examiner is invited to contact the applicant's undersigned representative if it is believed that a conference might further the prosecution of this matter.

Respectfully submitted,

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